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A Review of the Current Status of Running Buffalo Clover (*Trifolium stoloniferum*) in Ohio

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Abstract: Running Buffalo Clover, *Trifolium stoloniferum*, is a federally protected plant with two populations in Ohio. Although it had been generally accepted as extinct, populations were found in Ohio in 1985. Habitat losses, reduced soil disturbance, and invasive species have been major causes of declines in Running Buffalo Clover (RBC). This study investigated the status of RBC and provided additional evidence on several species impacting RBC's survival. Closely associated species are not well understood; however, endangered species of sage, bison, and elk are likely the most important associated species for RBC's survival, while white-tailed deer, rabbits, and cattle are detrimental. Several findings in this report support an important association with bison or elk. Root nodules are lacking in RBC, though they are present in closely related species of clover. Bison and elk manure is high in nitrogen, and RBC has low organic nitrogen-fixing abilities. There is an inverse relationship between available nitrogen and plants' nitrogen fixation capabilities. RBC requires a soil disturbance frequency that seems similar to bison migration frequencies. Recovery effort plans are now nine years old and current information about the plant's distribution and recovery is lacking. RBC still experiences declines in remaining areas and is in need of more robust management plans. Future investigations should focus on discovery of other suitable areas in Ohio for introduction, genetic analysis of RBC's ability to resist parasites, and the importance of bison and elk to RBC seed germination and survival.

Key Words: Running Buffalo Clover, *Trifoflium stoloniferum*, endangered plant, Ohio

Introduction

Running Buffalo Clover (RBC), *Trifoflium stoloniferum*, is an endangered plant found in a few fragmented locations in West Virginia, Indiana, Kentucky, and southwestern Ohio (US Fish and Wildlife Service, 2007). RBC is a perennial legume that produces runners with trifoliate leaves extending from one rooted crown system (Campbell et al., 1988). Its small white flowers appear in April and last until about June, resembling the common white clover (USFWS, 2007). RBC's natural habitat includes forest edges, clearings, and trails where there is some sun exposure (Bartgis, 1985; Cusick, 1989). RBC also requires elevated ground with layers of limestone underneath the soil (Andreas, Mack, and McCormac, 2004; Madarish and Schuler, 2002). Shortly after the arrival of European settlers in the Midwest, RBC began to decline due to habitat loss, elimination of disturbances, loss of associated species, invasive species, and possible diseases (Aldrich, Bacone, and Homoya, 1986; Brooks, 1983; Madarish and Schuler, 2002; USFWS, 2007). RBC was considered to be extinct, but in the mid-1980s, small populations were found in Ohio and in surrounding states (Bartgis, 1985). Unlike many legumes, RBC has low nitrogen-fixing capabilities and therefore grows in slightly acidic soil and may require large herbivores for effective seed germination (Morris et al., 2002). Disturbances produced by large herbivores, occasional mowing, logging, and fires are also important in sustaining the species as well as preventing invasive species or more competitive plants (Homoya, Aldrich, and Jacquart, 1989; Madarish and Schuler, 2002). Although Campbell et al. (1988) and Cusick (1989) claimed that RBC was reliant on American bison (*Bison bison*), there is little evidence to support this claim.

RBC is listed as endangered at federal and state levels and much attention has been focused on recovery efforts (Madarish and Schuler, 2002). RBC still requires continued management and monitoring (Scott et al., 2010) as evidenced by the Ohio Natural Heritage Program's (ONHP, 2007) finding that RBC populations have continued to decline in Ohio. With the USFWS (2007) recovery plan now nine years old, there is a need for current information on the status of RBC's population size, distribution, and the effectiveness of recovery plans for Ohio and other states within RBC's historic range. The purpose of this paper is to review the current status of RBC in Ohio and investigate important associated species.

Discussion

In Ohio, RBC was common in eight counties, but currently only two populations exist across three counties (ONHP 2007). RBC has the ability to penetrate the tough, compacted soil that characterizes much of Ohio and can convert the soil to a higher-quality soil (Madarish and Schuler, 2002). RBC also has the ability to withstand soil disturbances and droughts, making it an important part of pastures, where it can provide a food source for production animals (Morris et al. 2002; Taylor et al., 1994). RBC has a natural resistance to nematode infections that most cultivated clovers lack, making it agriculturally significant and an interesting subject for pathogen study (Quesenberry et al., 1997). RBC is an integral part of its ecosystem (Singha, Baker, and Bhatia, 1988) because of its important association with other plant species such as sedge, white snakeroot, and deer-tongue (Madarish and Schuler, 2002).

Current Threats. Habitat destruction began in the 1700–1800s, when settlers cleared almost all of RBC's native range for agriculture and timber (Brooks, 1983; Campbell et al., 1988; USFWS, 2007). Cully, Cully, and Hiebert (2003) estimated that only 1% of the original North American prairie remains. This area was RBC's historic range and also supported vast herds of bison and elk. Remaining habitat is highly fragmented and faces ongoing threats from continued development and the addition of invasive species (Allen, 1994; Burkhart, Rentch, and Schuler, 2013; Morris et al. 2002). This loss of key habitat has been cited as one of the main causes of decline in RBC and also played a role in the extirpation of other associated species (Campbell et al., 1988; Cusick, 1989; Ford et al., 2003; Morris et al., 2002; USFWS, 2007). Disease has been cited as a contributor to RBC's decline (Bartgis, 1985), yet this seems speculative because no evidence of disease has been discovered in wild populations (USFWS, 2007). Mildew has been found growing on RBC, though it had little effect on survival (Campbell et al., 1988). Still, disease should not be excluded from the list of threats given the low genetic diversity of RBC populations (Hickey, Vincent, and Guttman, 1991; Kongkiatngam et al., 1995).

Regular habitat disturbances are vital for reducing RBC's competitors, such as trees and other secondary growth (Burkhart, Rentch, and Schuler, 2013; Cully, Cully, and Hiebert, 2003; Madarish and Schuler, 2002). The frequency and intensity of these habitat disturbances are important, though the measures have not been firmly established. Timber forests, typically cleared about once every 10 years, were found to have several populations of RBC growing along the logging trails (Madarish and Schuler, 2002). Logging has been reduced in Ohio, leading to a decrease in disturbances and an increase in competitors (Burkhart, Rentch, and Schuler, 2013; Pavlovic, 1994). Following the reduction in logging, the Ohio Department of Natural Resources estimates that Ohio's forest cover has improved from a low of 15% in 1940 to more than 30% by 1994 (ODNR, 1994). While the increase in forest cover may be hailed as an environmental success, it coincides with the recent declines in RBC populations. The loss of the disturbance events may also have left the soil less compacted, to the detriment of RBC, but allowing other species to dominate the area (Burkhart, Rentch, and Schuler, 2013).

Closely Associated Species. The relationships and importance of closely associated species and RBC are not well understood, though several are described. Madarish and Schuler (2002) found that sedge, white snakeroot, hog peanut, and deer-tongue were often found with RBC. According to the ODNR (2013a), more than 55 species of sedge and 30 species of grasses are in decline, which may contribute to RBC's decline. Interestingly, Morris et al. (2002) reported that deer-tongue, which is not endangered, was always found among the areas with RBC. Herbivores play an integral role in RBC's life cycle, but no actual evidence has been provided to support any type of special relationship with any particular herbivore (Campbell, 1988; Cusick, 1989). Establishing which species of herbivores are important associates has been difficult. Large herbivores, such as elk and bison, were thought to be important, but by 1850, both species had been extirpated throughout RBC's range (Cox, 2011; Ford et al., 2003; ODNR, 2008). Research suggests that large herds of elk and bison grazing on RBC across large migratory routes likely provided ideal conditions with regular disturbances, effective seed dispersal, and manure that was high in nitrogen (Cox, 2011; Homoya, Aldrich, and Jacquart, 1989; Madarish and Schuler, 2002; Morris et al., 2002). Additional evidence comes from a comparison of RBC to other closely related species. Unlike closely related clovers, RBC has poor nitrogen-fixing abilities and lacks root nodules (Morris et al., 2002). In contrast, red clover (*Trifolium pratense*) has root nodules and is efficient at nitrogen fixation (Sturz et al., 1997). Large herbivores have shown a strong preference for RBC (Miller, Bratton, and Hadidian, 1992) and their manure is high in nitrogen, vital to RBC's survival (Morris et al., 2002). Ledgard and Steele (1992) reported that plants' nitrogen fixation ability decreases as the concentration of nitrogen in the soil increases. It is likely that RBC evolved reduced nitrogen-fixing abilities as large herbivores' manure provided an abundant source of nitrogen.

With the extirpation of large herbivores, populations of smaller herbivores such as white-tailed deer and rabbits increased (Ford et al., 2003; Cox, 2011). Since 1970, the white-tailed deer population in Ohio has grown from 17,000 to 750,000 (ODNR, 2013b) and the population of Eastern cottontail rabbits is far larger now than before settlers arrived (ODNR, 2014). While elk and bison migrate across wide ranges, white-tailed deer, rabbits, and cattle remain in much smaller areas (Cox, 2011; Ford et al., 2003; USFWS, 2007). In contrast, longer migrations of elk and bison may have provided wide seed dispersal and the correct frequency of disturbances (Burkhart, Rentch, and Schuler, 2013; Ford et al., 2003; USFWS,

2007). Although white-tailed deer and rabbits have been proposed as replacements for large herbivores (Cusick, 1989), other evidence refutes this. Campbell (1988) found that RBC declined when rabbits and white-tailed were allowed to graze in the areas. RBC sites that were grazed by cattle also experienced declines, likely due to their intense and destructive grazing habits (Burkhart, Rentch, and Schuler, 2013; Pavlovic, 1994). The highly intense grazing habits of cattle, white-tailed deer, and rabbits may have selected for more competitive clovers or invasive species (Ford et al., 2003). Additionally, RBC seeds consumed by white-tailed deer showed decreased rates of germination and survival, indicating a specific vector for seed germination (Ford et al., 2003). RBC germination requires scarification of the outer seed coat (Campbell et al., 1988; Morris et al., 2002). It is possible that only the dentition of large herbivores or the more extensive digestive systems of bison and elk provides enough scarification along with adequate time in an acidic environment for RBC seed germination (Ford et al., 2003).

Recovery Efforts. Recovery efforts for existing populations are focused around the Cincinnati area and Wayne National Forest in southeast Ohio (USFWS, 2007). The former is protected and managed by the ODNR and Ohio Historical Society and the latter is managed by ODNR (USFWS, 2007). After successful reintroductions of RBC in the 1980s, a drastic decline occurred in 2005, possibly from an unknown disease and overgrazing (USFWS, 2007). In rare plant reintroduction efforts, initial successes followed by sudden collapses are common and the causes are usually unknown (Allen, 1994). Although disease cannot be completely ruled out, evidence to support the claim has not been found. The most plausible cause of recent RBC declines is overgrazing by white-tailed deer and rabbits. White-tailed deer and Eastern cottontail rabbits were most numerous in counties where RBC populations are found, and both were at record highs between 2003-2005 when the RBC populations declined (ODNR, 2011; 2014). Miller, Bratton, and Hadidian (1992) found that white-tailed deer showed a preference for RBC and quickly decimate populations. Given that the highest threat to survival seems to be from white-tailed deer and rabbits, recovery efforts must eliminate these herbivores or restrict their access to RBC locations.

Selecting additional recovery sites may improve the overall effectiveness of conservation efforts for RBC. Elevation and the presence of limestone formations are characteristic of the soil in areas around west and central Ohio (Ohio Division of Geological Survey, 2006). There may be several areas that could be ideal RBC habitat and support reintroductions. Another option is to reintroduce RBC to areas with existing herds of bison or elk. Cox (2011) referenced several isolated RBC individuals at elk reintroduction sites in Kentucky. Reintroductions at these sites could increase the number of individuals and eventually form another population.

Combining Efforts to Improve Conservation. While not all plants can produce roots from cut stems, RBC has shown the ability to produce new roots in artificial media (Singha, Baker, and Bhatia, 1988). This makes RBC an ideal candidate for research at the Cincinnati Zoo's Carl H. Lindner Jr. Family Center for Conservation and Research of Endangered Wildlife (CREW, n.d.). The facility is located in Cincinnati, Ohio, and specializes in rare species on the brink of extinction. Specimens at this facility are preserved in artificial media and kept in long-term storage. A closely related native species, *Trifolium calcaricum*, has had similar declines, yet no specific causes were determined (Collins and Wieboldt, 1992). Similarities exist between RBC and *T. calcaricum*, especially in terms of nitrogen fixation, and conservation efforts could be combined.

Understanding the importance of associated plant and animal species has been difficult because wild populations of RBC are often rare and literature is lacking. While there is no information to indicate that research is being done on RBC at sites where large herbivores have been reintroduced, it could be an opportunity to investigate and monitor them in their natural environment. Studies of elk reintroduced to abandoned strip mines in Kentucky (Cox, 2011) could be combined with RBC investigations because there is an important association between the two species. This area contains suitable habitat and isolated individuals have been observed (Cox, 2011; USFWS, 2007). The Wilds is a10,000-acre park owned by the Columbus Zoo and Aquarium (2012), located in Cumberland, Ohio. The park has a herd of bison (Columbus Zoo and Aquarium, 2012) and contains areas that meet RBC's habitat requirements (Ohio Division of Geological Survey, 2006). The park may be able to support recovery efforts of RBC and provide an opportunity for additional research into the relationship of bison and RBC.

Future Investigation. All known populations of RBC exist in the southwest corner of Ohio and in the Wayne National Forest (USFWS, 2007). Future investigation should focus on finding new populations and new areas for reintroduction. Allen (1994) provided hopeful information about reintroduction through transplantation; however, there is no data to evaluate the long-term effectiveness of this method. USFWS (2007) also discussed reintroduction, but specific methods and successful long-term results were absent. Many of Ohio's counties have significant elevation and are rich in limestone (Ohio Division of Geological Survey, 2006), which may support unknown populations or could host reintroductions. Ohio has many abandoned coal mines in counties near existing populations of RBC (Harris, 2009). The existence of elk and bison in neighboring states creates an opportunity to evaluate the shared successes and investigate further reintroductions. A shared conservation project between these states could also provide an opportunity to investigate the importance of endangered species and closely associated

species. RBC is an ideal plant for researchers at the Cincinnati Zoo CREW facility, though no information could be obtained to determine if this has already been done. Further investigation is needed to understand the genetics and mechanisms of RBC's natural pest resistance. If this property could be better understood, it could lead to a reduction in the use of agricultural pesticides. The threat of disease must still be considered because remaining populations of RBC have low genetic variation and may have no resistance to current or future diseases (Hickey, Vincent, and Guttman, 1991; Kongkiatngam et al., 1995). The low genetic diversity of RBC identified by Kongkiatngam et al. (1995) will continue to be of concern and requires continued monitoring of remaining populations.

Conclusions

Running Buffalo Clover is an endangered plant that exists in fragmented populations across its former range. It is an important species for agriculture as well as a resource that can provide insight for agricultural pest management. Main threats include habitat loss, reduction of disturbances, and invasive species (Madarish and Schuler, 2002). Within Ohio, two areas are home to the remaining populations and are the focus of recovery efforts. Newer insight and compiled evidence supports that white-tailed deer and rabbits are a larger threat than previously thought. Current declines in remaining RBC populations are likely caused by the rapid growth of Ohio's white-tailed deer and rabbit populations. Several findings in this report support an important association with bison or elk. Root nodules are lacking in RBC, though they are present in closely related species of clover. Bison and elk manure is high in nitrogen and RBC has low organic nitrogen-fixing abilities. There is an inverse relationship between available nitrogen and plants' nitrogen fixation capabilities. RBC requires a specific soil disturbance frequency that could be provided by migratory herbivores such as bison and elk; however, intense, destructive grazing by white-tailed deer, rabbits, and cattle is detrimental. Sedge, deer-tongue, bison, and elk have important close associations with RBC and the effect of closely associated species is more critical to the survival of RBC than previously thought. The loss of these closely associated species has been the most significant factor in RBC's decline. Cattle, white-tailed deer, and rabbits should be restricted from all recovery sites. RBC's low genetic diversity is a concern, though disease still does not seem to pose an immediate threat. More work is needed in combining RBC conservation efforts with other endangered species. This would benefit multiple species and foster understanding of the nature and importance of closely associated species. Future investigations should seek to evaluate the status of reintroduction efforts, the frequency and intensity of disturbances, and the genetics and mechanisms of parasite resistance. After a thorough review of the literature, information on current distribution and status of recovery of RBC is lacking.

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Short-Term Effects of a Prescribed Burn on Butterfly Abundance and Diversity in a Restored Northeastern Ohio Prairie

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Abstract: Prescribed burns are a common management technique used across the globe and are often used to control encroaching vegetation in prairie systems. Although effective for managing vegetation, fire can have variable effects on insects such as butterflies and is generally linked to disruption of reproductive cycles and host plant use. This study was conducted to determine the effects of a prescribed burn on adult butterflies within a short time scale (3 months post-burn). Surveys were conducted in July 2013 (prior to the burn) and July 2014 (following the burn) using a combination of plot and transect surveys. Overall, both total abundance and species richness declined significantly following the prescribed burn. Responses, however, were species-specific, with three major outcomes: 1) some species were detected in the summer prior to the burn but not following it; 2) some species were detected during the summer following the burn but not prior to it; and 3) some species were found during both years. Pearl crescents (*Phyciodes tharos*), common butterflies in this area, significantly decreased in abundance following the burn. Further, great spangled fritillaries, orange/clouded sulphurs, and cabbage whites, all common generalist species, were not detected during surveys following the burn. These four common species were, however, detected in the control plot and during transect surveys. Although not all butterflies were negatively affected, fire did adversely impact the community as a whole. As insect pollinators continue to decline, prairie managers should consider using alternative methods such as haying and grazing when possible. However, when fire is used, nearby refugia and host plants should be set aside and conserved for sensitive species.

Keywords: Lepidoptera, fire, disturbance, pearl crescents, population, species richness

Introduction

Globally, insect pollinators have been in decline over the last several decades. Population losses have been linked to habitat loss and fragmentation, use of agrochemicals, the spread of pathogens, introduction of alien species, and climate change (see Potts et al., 2010 for a review). Because pollinators provide essential ecosystem services and play a key role in both the ecological landscape and human society, conservation measures to protect insect pollinators have gained increasing awareness through initiatives such as the Convention on Biological Diversity's International Pollinator Initiative (http://www.cbd.int/decision/cop/?id=7147), and through citizen science programs such as Bumble Bee Watch (http://www.bumblebeewatch.org/) and Monarch Watch (http://www.monarchwatch.org/). Although most studies have focused on the decline of the European honeybee (*Apis mellifera*), many other pollinator species are in peril, including prairie-specialist butterflies, which have shown rapid population declines over the last century (Orwig, 1992; Schlicht and Orwig, 1998).

It is estimated that approximately 98% of North American tallgrass prairie has been destroyed (primarily for agricultural use) since European establishment in North America (Drache, 2001), and this has likely been a major cause in the decline of prairie-specialist butterflies. As a result, prairie restoration projects have become common across the Midwest and are often managed by controlled burns, mowing/haying, and/or grazing to maintain prairie vegetation, prevent forest succession, and control the establishment of invasive species. Previous studies have shown that butterfly populations can benefit from prescribed burns (Huntzinger, 2003; Panzer and Schwartz, 2000) or that prescribed burns have no net effect on species diversity (Fleishman, 2000). However, even if diversity is not influenced by burning, certain species may be affected differently and some management practices may favor some butterfly species while negatively affecting others (Vogel et al., 2007). Conversely, there are several studies that show declining trends of butterfly abundance and/or diversity following a prescribed burn (Swengel, 1996; Vogel et al., 2010; Swengel et al., 2011) with recovery taking approximately three to six years or more (Swengel, 1996; Vogel et al., 2010).

In this study, we surveyed butterfly populations within a restored prairie in northeastern Ohio prior to and approximately three months after a prescribed burn. Surveys were conducted weekly during the month of July, when butterfly activity was at its peak. Additional surveys were conducted in a nearby Monarch Waystation as a control, and transect surveys were completed

in surrounding habitats post-burn to compare species presence and absence. Although the literature suggests that the effect of prescribed burns on abundance and diversity is not consistent across study sites, we predicted that burning would decrease both plant diversity and butterfly abundance and diversity in the short term.

Methods

Field site description. All butterfly surveys were conducted at the James H. Barrow Field Station, Hiram College, Portage County, Ohio (41.299521 N, -81.109085 W). This field station covers approximately 380 acres of land and is comprised of beech-maple forest, wetlands, streams, and successional old field meadows. In 2008, approximately three acres (140,000 sq. ft.) of continuously mowed grass were set aside for two major reasons: 1) to construct a zig-zag wetland to purify greywater from field station buildings and 2) to allow for natural succession of meadow vegetation. In 2011, a native tallgrass prairie mix including both grasses and forbs (packaged by Ohio Prairie Nursery) was planted in an effort to mimic the native prairies historically found in northeastern Ohio. Following the planting in 2011, native seed has been scattered and additional plugs of both grasses and forbs have been planted throughout the area. In April 2014, the first prescribed burn was completed over the entire prairie for management purposes.

In 2009, a Monarch Waystation (approximately 200 m^2 in size) was constructed and certified according to Monarch Watch's Waystation standards. Although this garden was established to attract monarchs, several flowering species have been planted to increase visitation of butterflies in general. This garden is situated approximately 70 m from the restored prairie. Due to the close proximity and the lack of disturbance during the study, this garden was used as a control site for the restored prairie.

Butterfly surveys. A 200 m^2 plot was established in the restored prairie during the summer of 2013 (equal to the size of the Monarch Waystation). Butterfly surveys were conducted weekly (N = 4 per site) throughout the month of July in both 2013 (pre-burn) and 2014 (post-burn) within both the restored prairie and the Monarch Waystation. Plots were surveyed between 10 a.m. and 4:00 p.m. on sunny days with winds no stronger than five on the Beaufort wind scale. Timed surveys were conducted using four observers stationed within the plot for a duration of 20 minutes. To catalog both abundance and diversity, butterflies were captured with butterfly nets and held in jars until the end of the survey period. Individuals not captured were identified in flight and special care was taken to estimate numbers conservatively to avoid re-counting butterflies missed by netters. All butterflies were identified to the species level and released live following surveys.

In addition to plot-style surveys, transect surveys were conducted during July 2014, on the same days as plot surveys, to allow for a larger area of coverage and with the goals of surveying as many butterfly species as possible and detecting the presence of species that may have not visited the restored prairie and/or Monarch Waystation. The transect was mapped out across the James H. Barrow Field Station in accordance to standards set by the Ohio Lepidopterists Society for long-term monitoring (http://www.ohiolepidopterists.org/bflymonitoring/instructions/default.htm). This transect represented a variety of habitats, including successional old fields, forests, and wetlands, and took approximately 45 minutes to walk at a moderate pace. All butterflies within 15 feet of the recorder were identified and counted.

Flowering plant surveys. Plant surveys were conducted twice during July 2013 within the 200 m^2 plot used for butterfly surveys in the restored prairie. In 2013, stem counts were completed for all plants that were in flower and plants were identified to species. During July 2014, a visual inspection was completed during butterfly surveys in the restored prairie to catalog species in flower and determine percent cover.

Statistical analyses. One-way ANOVAs were used to compare differences between July 2013 and 2014 for butterfly abundance and species richness, pearl crescent abundance, and flowering plant species richness (JMP 10.0.0, SAS Institute Inc., 2012). One flowering plant species was unable to be identified and was cataloged as an unknown.

Results

Butterfly surveys. Both total abundance (2013 average = 8.75 ± 2.32 [mean ± 1SE], 2014 average = 3.75 ± 0.25, ANOVA, $F_{(1,6)} = 4.58$, $P = 0.0761$) and species richness (2013 average = 2.75 ± 0.25 [mean ± 1SE], 2014 average = 3.5 ± 0.28, ANOVA, $F_{(1,6)} = 3.86$, $P = 0.0972$) were not significantly different for the Monarch Waystation between July 2013 and 2014. Although many of the species that visited the Monarch Waystation across these two summers were similar, there were some butterflies that were detected in 2013 but not in 2014 (Table 1). Following the prescribed burn, both total abundance (ANOVA, $F_{(1,6)} = 72.98$, $P < 0.0001$) and species richness (ANOVA, $F_{(1,6)} = 12.00$, $P = 0.0134$) decreased significantly (Figs. 1a and b). In addition

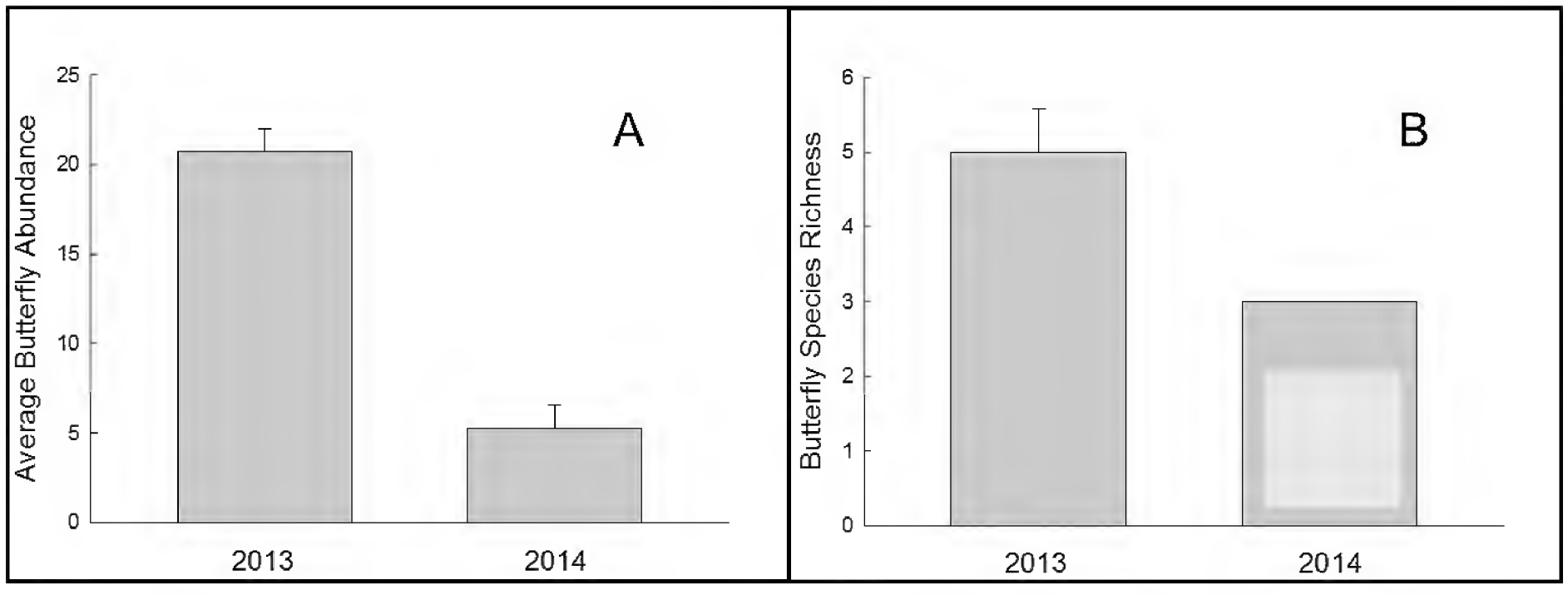

Figure 1. Average butterfly abundance (a) and species richness (b) (mean ± 1 SE) at the restored prairie from July 2013 (prior to the prescribed burn) and 2014 (following the prescribed burn) surveys.

to declines in overall abundance, pearl crescent (*Phyciodes tharos*) butterfly abundance (also an incredibly common butterfly species) decreased significantly following the prescribed burn (2013 average = 14.75 ± 1.65 [mean ± 1SE], 2014 average = 2.75 ± 1.55, ANOVA, $F_{(1,6)} = 28.10$, $P = 0.0018$). Great spangled fritillaries (*Speyeria cybele*), orange/clouded sulphurs (*Colias eurytheme/Colias philodice*), and cabbage whites (*Pieris rapae*) (three very common species at the James H. Barrow Field Station) were detected prior to the prescribed burn, but were not detected following the burn (Table 2). These three species were, however, detected in July 2014 in the nearby Monarch Waystation (Table 1) and during transect surveys (Table 3). Eastern tailed-blue (*Everes comyntas*) and mourning cloak (*Nymphalis antiopa*) butterflies were detected prior to the prescribed burn, but were absent from all surveys in July 2014. Although many common species were not detected following the prescribed burn, four species absent during July 2013 surveys were found in 2014: silver-spotted skipper (*Epargyreus clarus*), cobweb skipper (*Hesperia metea*), little sulphur (*Eurema lisa*), and little wood satyr (*Megisto cymela*). All but the cobweb skipper were detected during transect surveys.

Table 1. Butterfly species detected at the Monarch Waystation during July 2013 and 2014 surveys. The "X" indicates presence during that year.

Butterfly Species	July 2013	July 2014
Orange/clouded sulphur (*Colias eurytheme/C. philodice*)	X	X
Pearl crescent (*Phyciodes tharos*)	X	X
Great spangled fritillary (*Speyeria cybele*)	X	X
Summer azure (*Celastrina neglecta*)	X	X
Cabbage white (*Pieris rapae*)	X	X
Eastern tiger swallowtail (*Papilio glaucus*)	X	
Horace's duskywing (*Erynnis horatius*)	X	
Banded hairstreak (*Satyrium calanus*)		X
Crossline skipper (*Polites origenes*)		X
Meadow fritillary (*Boloria bellona*)		X
Edward's hairstreak (*Satyrium edwardsii*)		X
Common wood nymph (*Cercyonis pegala*)		X
Little wood satyr (*Megisto cymela*)		X
Sachem (*Atalopedes campestris*)		X
European skipper (*Thymelicus lineola*)		X

Table 2. Butterfly species detected at the restored prairie prior to the prescribed burn (July 2013) and following (July 2014). The "X" indicates presence during that year.

Butterfly Species	July 2013	July 2014
Orange/clouded sulphur (*Colias eurytheme/C. philodice*)	X	
Great spangled fritillary (*Speyeria cybele*)	X	
Cabbage white (*Pieris rapae*)	X	
Eastern tailed-blue (*Everes comyntas*)	X	
Mourning cloak (*Nymphalis antiopa*)	X	
Pearl crescent (*Phyciodes tharos*)	X	X
Eastern tiger swallowtail (*Papilio glaucus*)	X	X
Meadow fritillary (*Boloria bellona*)	X	X
Common wood nymph (*Cercyonis pegala*)	X	X
Silver-spotted skipper (*Epargyreus clarus*)		X
Little wood satyr (*Megisto cymela*)		X
Cobweb skipper (*Hesperia metea*)		X
Little sulphur (*Eurema lisa*)		X

Flowering plant surveys. Visual inspection during butterfly surveys indicated that red clover (*Trifolium pretense*, approximately 95% cover) and black-eyed Susans (*Rudbeckia hirta*) were the only two species present that were in flower during the month of July. Flowering plant species richness was significantly higher during July 2013 (2013 average = 8.5 ± 0.5 [mean ± 1SE], 2014 average = 2.0 ± 0, ANOVA, $F_{(1,2)} = 169.00$, $P = 0.0059$) (see Table 4 for a complete listing of plants found during 2013 survey).

Table 3. Butterfly species detected through transect surveys during July 2014.

Butterfly Species July 2014
Orange/clouded sulphur (*Colias eurytheme/C. philodice*)
Pearl crescent (*Phyciodes tharos*)
Great spangled fritillary (*Speyeria cybele*)
Summer azure (*Celastrina neglecta*)
Cabbage white (*Pieris rapae*)
Eastern tiger swallowtail (*Papilio glaucus*)
Meadow fritillary (*Boloria bellona*)
Common wood nymph (*Cercyonis pegala*)
Little wood satyr (*Megisto cymela*)
Silver-spotted skipper (*Epargyreus clarus*)
Little sulphur (*Eurema lisa*)
Black swallowtail (*Papilio polyxenes*)

Table 4. Flowering plants surveyed during July 2013 (prior to the prescribed burn).

Flowering Plant Species July 2013
Queen Anne's lace (*Daucus carota*)
Black-eyed Susan (*Rudbeckia hirta*)
Red clover (*Trifolium pretense*)
Oxeye daisy (*Leucanthemum vulgare*)
Prairie coneflower (*Ratibida pinnata*)
Eastern purple coneflower (*Echinacea purpurea*)
Henbit (*Lamium amplexicaule*)
Woodland sunflower (*Helianthus divaricatus*)

Discussion

Surveys from this study showed that both butterfly abundance and diversity declined following the prescribed burn. Because abundance and diversity did not differ between 2013 and 2014 in the nearby Monarch Waystation and common butterflies missing from the restored prairie were detected during transect surveys, we can conclude that the effects of fire in the short

term were an important cause of declines in the restored prairie. These results are consistent with other studies in which overall abundance declined on a short time scale following a burn (Panzer, 2002; Swengel and Swengel, 2007). However, this trend is not consistent across geographical locations and systems. Some studies show that fire can increase butterfly abundance and diversity (Taylor and Catling, 2012; Huntzinger, 2003; Verdasca et al., 2012; Scandurra et al., 2014), while sometimes it has no net effect on diversity (Fleishman, 2000). Further, responses to fire can be species-specific, with some butterfly species experiencing negative effects and others benefitting from this disturbance (Swengel, 1996; McIver and Macke, 2014). Similarly, surveys from this study also showed that butterflies can have species-specific responses to fire. Because the prescribed burn had marked effects on the flowering plant community, we suggest that this may be linked to overall declines and species-specific responses.

Cool-season fires typically favor several dominant, native, warm-season grasses, which, in turn, reduce the abundance and diversity of forbs, especially when burns are frequent (Ewing and Engle, 1988). Further, plants that grow primarily in the cool season are more likely to decline after a fire occurring in that same season (Ewing and Engle, 1988). In this study, flowering plant diversity declined drastically post-burn; red clover was the main plant in flower, along with a small number of black-eyed Susans. Since many butterfly specialists feed primarily on forb species (Davis et al., 2008), this may explain the decrease in abundance and diversity of butterflies following this cool-season prescribed burn.

In this study, four common species were found either in lower abundance following the burn (pearl crescent) or were completely absent (great spangled fritillary, orange/clouded sulphur, and cabbage white). These four species were found during surveys in other nearby locations at the James H. Barrow Field Station during 2014, suggesting that they respond negatively to fire. Pearl crescents, great spangled fritillaries, orange/clouded sulphurs, and cabbage whites are all considered to be habitat generalists with regard to both habitat use and adult nectar sources. Great spangled fritillaries and cabbage whites are known to use red clover as a nectar source and were expected visitors at this site. This reduction of generalist species is inconsistent with many studies that show that specialist species are more negatively impacted by fire management than species that are more common (see Swengel, 2011, for a review). Generalist butterfly species with broad habitat niches tend to recover more quickly following a burn than specialist butterfly species, especially if source populations and host plants are nearby (Swengel, 1996). Vagility and voltinism can affect the recovery of butterfly populations, with multivoltine species colonizing more readily following fire (Swengel 1996).

Conversely, our surveys showed that four species were found during post-burn surveys that were not detected during pre-burn surveys: silver-spotted skipper, cobweb skipper, little sulphur, and little wood satyr. Adult cobweb skippers have been found in association with burned landscapes and habitats with *Andropogon* spp., the larval host plant for this species (Shapiro, 1965). *Andropogon gerardi* (big bluestem) is prevalent throughout the restored prairie in this study and recovered quickly following the prescribed burn. Cobweb skippers are considered to be fire-adapted because the first instars feed nocturnally underground at the base of plants where they are protected from ground fires (Shapiro, 1974). Since silver-spotted skippers (Stichter, 2015) and little sulphurs (Glassberg, 1999) are well adapted to disturbances, they may have been able to recover more quickly and/or were visitors from nearby refugia. Further, the little wood satyr was found throughout the James H. Barrow Field Station in 2014, and nearby refugia likely contributed to its visitation to the prescribed burn plot.

Lastly, three species (the Eastern tiger swallowtail, meadow fritillary, and common wood nymph, all common species in northeastern Ohio) were present during surveys for both years, suggesting that the burn did not have a substantial effect on these species. Further, all three species were also found in high abundance during July transect surveys. Host plants for these species are evenly distributed throughout the James H. Barrow Field Station property and this likely accounts for their visitation to the prescribed burn plot.

Although this study showed species-specific responses, butterfly populations as a whole did not recover within a growing season, which is likely linked to decreased plant diversity and direct mortality. Swengel (1996) suggests that for populations that experience declines following a burn, recovery can take at least 3–5 years. However, it is common for newly established prairies to be burned every year and then every 3–5 years once vegetation is well established. Although fire may benefit some species, this prescription likely does not allow enough time for many butterfly species to recover. In general, fire has negative impacts on insect communities (see Swengel, 2001, for a review), but some taxa appear to recover quickly (within one to two years), especially those with high vagility (Panzer, 2002).

There is growing concern among entomologists that prairie insect species may be threatened by prescribed burning practices. Since this management tool can result in a substantial loss of insect species richness, several authors recommend that the use of prescribed burns should be minimized (Orwig, 1992; Swengel, 1996). As pollinators continue to decline, managers should consider using other methods such as haying, grazing, and brush-cutting to manage prairie plants. Areas formerly

burned may take six to eight years to become refuge habitat for butterflies (Swengel and Swengel, 2007), and using multiple methods within a landscape would likely be less harmful to butterfly populations, as species respond differently to management practices (Swengel, 1996). Further, areas should be set aside as a refuge when these management techniques are implemented. In landscapes that are burned periodically, refugia offer persistent habitat diversity where organisms adverse to fire are more likely to survive (York, 1999; Swengel and Swengel, 2007).

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